

In the Claims

Claim 1 (currently amended). A method of concentrating, detecting, and extracting particles from a whole blood sample, the method comprising:

exposing the blood sample to an enzyme-detergent combination; and
analyzing the exposed blood sample for the presence of particles.

Claim 2 (original). The method of claim 1 wherein the enzyme-detergent combination comprises plasminogen and streptokinase.

Claim 3 (currently amended). The method of claim 2 further comprising the steps of:
maintaining~~freezing~~ the plasminogen and streptokinase in a frozen state in coincident relation until exposure of the blood sample to the enzyme-detergent combination~~a fibrin lysis reagent is needed~~; and

reacting streptokinase with plasminogen upon thawing whereby plasmin is formed.

Claim 4 (currently amended). The method of claim 3 wherein~~2 further comprising the step of~~ suspending the plasminogen is suspended in an aqueous salt solution prior to freezing.

Claim 5 (original). The method of claim 4 wherein the aqueous salt solution comprises NaCl.

Claim 6 (currently amended). The method of claim 4 wherein the aqueous salt solution comprises Na₃PO₄~~NaPO₄~~.

Claim 7 (currently amended). The method of claim 1 wherein the particles are selected from the~~a~~ group consisting of prions, toxins, metabolic markers, cancerous matter, disease state markers, bacteria, virus, and fungi.

Claim 8 (currently amended). The method of claim 1 wherein the particles are DNA molecules and further comprising the step of replicating the particles through PCR.

Claim 9 (currently amended). The method of claim 1 further comprising the step of introducing DNase to exposing the blood sample to a DNase.

Claim 10 (currently amended). The method of claim 1 further comprising the step of introducing Endonuclease to exposing the blood sample to an endonuclease.

Claim 11 (original). The method of claim 2 wherein the plasminogen and streptokinase are in a dried state.

Claim 12 (original). The method of claim 11 wherein the plasminogen and streptokinase are mixed and distributed in disposable test containers.

Claim 13 (currently amended). The method of claim 11 wherein the plasminogen is combined with one or more enzymes selected from the group consisting of Phospholipase A₂, DNase, Endonuclease, and Lipase.

Claim 14 (original). The method of claim 13 wherein the enzyme-detergent combination is suspended then dried in pellets of trehalose buffer and packaged as a dry reagent.

Claim 15 (original). The method of claim 11 wherein the streptokinase is suspended then dried in pellets of trehalose buffer and packaged into tubes as a dry reagent.

Claim 16 (currently amended). The method of claim 11 further comprising:
resuspending the dried reagents plasminogen and streptokinase in a buffer solution;
adding the buffer solution containing plasminogen and streptokinase to the volume of blood;

and

incubating the blood sample for at room temperature.

Claim 17 (currently amended). The method of claim 16, wherein the ~~dried reagent is comprised of enzymes of the enzyme-detergent combination are in a dried state and comprise 1,500-4,500 KU Phospholipase A₂, 5,000-10,000 U Streptokinase, 2-10 U Plasminogen, 200-3,650 U DNase, 200-4,000 U Endonuclease, and 10,000-100,000 U Lipase.~~

Claim 18 (currently amended). The method of claim 16 further comprising: centrifuging the buffer solution to form a supernatant and a pellet; decanting the supernatant from the centrifuged buffer solution; and washing the pellet.

Claim 19 (currently amended). The method of claim 18 wherein the buffer solution is centrifuged for approximately 20 minutes at 5,000-5,500 x g at a temperature of 10-20°C.

Claim 20 (original). The method of claim 18 wherein the pellet is washed with an Ecotine-HEPES solution.

Claim 21 (original). The method of claim 18 wherein the pellet is washed with a Sucrose-HEPES solution.

Claim 22 (original). The method of claim 18 wherein the pellet is washed with an Ecotine-HEPES solution and a Sucrose-HEPES solution.

Claim 23 (currently amended). The method of claim 16 further comprising: centrifuging the buffer solution to form a supernatant and a pellet; decanting the supernatant from the centrifuged buffer solution; digesting the sample; and

applying the sample to a ~~commercially available~~ nucleic acid extraction method.

Claim 24 (original). The method of claim 23 wherein digesting the sample further comprises lysis and DNase inactivation.

Claim 25 (original). The method of claim 23 wherein digesting the sample further comprises lysis and Endonuclease inactivation.

Claim 26 (original). The method of claim 23 wherein digesting the sample further comprises utilizing proteinase K, sodium dodecyl sulfate, aurintricarboxylic acid, and sodium citrate buffer, incubated at room temperature.

Claim 27 (currently amended). The method of claim 16 further comprising:
filtering the buffer solution;
washing the buffer solution;
digesting the sample; and
purifying the nucleic acid from the digested sample ~~extract through commercially available methods.~~

Claim 28 (original). The method of claim 27 wherein digesting the sample further comprises lysis and DNase inactivation.

Claim 29 (original). The method of claim 27 wherein digesting the sample further comprises lysis and Endonuclease inactivation.

Claim 30 (original). The method of claim 27 wherein digesting the sample further comprises the steps of:

combining proteinease K, aurintricarboxylic acid, and sodium citrate buffer;
incubating at room temperature; and

eluting the lysate from the filter surface.

Claim 31 (currently amended). The method of claim 16 further ~~comprises~~comprising applying the solution directly to a biosensor device whereby responsive to the presence of the pathogens in the blood sample, the patient develops pathogenic or native disease state markers which allow for the capture and detection of these markers by the biosensor device.

Claim 32 (currently amended). The method of claim 16 further ~~comprises~~comprising applying the solution directly to a liquid chromatography mass spectrometry device whereby, responsive to the presence of the pathogens in the blood sample, the patient develops pathogenic or native disease state markers that allow for the detection of mass signatures associated with the structural components of the pathogens using the mass spectrometry device.

Claim 33 (original). The method of claim 16 wherein the buffer comprises Potassium Phosphate, Magnesium Chloride, Sodium Chloride, and Aurintricarboxylic Acid.

Claim 34 (currently amended). The method of claim 33 wherein the buffer further comprises Triton X-100~~octylphenol ethoxylate~~.

Claim 35 (currently amended). The method of claim 33 ~~further comprising the step of storing the enzymes with~~ wherein the enzyme-detergent combination comprises a trehalose buffer.

Claim 36 (currently amended). The method of claim 35 wherein further comprising the step of combining methyl 6-O-(N-heptylcarbamoyl)- α -D-glucopyranoside and Saponin are provided in the trehalose buffer.

Claim 37 (currently amended). The method of claim 36 wherein [[a]] the concentration of 20-35 mM ~~of~~ methyl 6-O-(N-heptylcarbamoyl)- α -D-glucopyranoside is used in the trehalose buffer is 20-35mM.

Claim 38 (currently amended). The method of claim 36 wherein [[a]] the concentration of ~~0.05-0.1%~~ Saponin ~~is used in the trehalose buffer~~ is 0.05-0.1%.

Claim 39 (currently amended). The method of claim 35 wherein the trehalose storage buffer comprises Potassium Phosphate, octylphenol ethoxylate~~Triton X-100~~, Dithiothreitol, and Trehalose.

Claim 40 (original). The method of claim 39 wherein the trehalose storage buffer comprises 10 mM Potassium Phosphate.

Claim 41 (currently amended). The method of claim 39 wherein the trehalose storage buffer comprises 0.01-0.04%~~Triton X-100~~octylphenol ethoxylate.

Claim 42 (original). The method of claim 39 wherein the trehalose storage buffer comprises 1-5 mM Dithiothreitol.

Claim 43 (original). The method of claim 39 wherein the trehalose storage buffer comprises 0.3-0.5 M Trehalose.

Claim 44 (new). The method of claim 1 wherein prior to exposing the blood sample to an enzyme-detergent combination the blood is contacted with an anticoagulant.

Claim 45 (new). The method of claim 1 wherein the blood exposed to the enzyme-detergent combination is unclotted whole blood.

Claim 46 (new). The method of claim 1 wherein the whole blood sample is at least 6.0 ml.

Claim 47 (new). The method of claim 1 wherein the enzyme-detergent combination comprises a Phospholipase A₂ and a DNase.

Claim 48 (new). The method of claim 47 further comprising exposing the blood sample to methyl 6-O-(N-heptylcarbamoyl)- α -D-glucopyranoside and Saponin.

Claim 49 (new). The method of claim 1 further comprising exposing the blood sample to a DNase and aurintricarboxylic acid.

Claim 50 (new). The method of claim 33 wherein the method is conducted at pH 7.8 to 8.0.